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Comparison of Allergenic Properties among Commercially Available House Dust Mite Allergen Extracts in Korea

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Precise allergy diagnosis and effective allergen specific immunotherapy are largely dependent on the quality of allergen extract. A new extract of *Dermatophagoides farinae* was commercially developed by Prolagen. The allergenic properties of the new extract were compared with those of other commercial products. The allergenic properties of the new extract were compared according to protein concentration, protein profiles, major allergen (Der f 1) contents, and allergenic potency to those for three commercially available extracts imported in Korea (Jubilant HollisterStier Allergy, Lofarma S.p.A., and Stallergenes Greer). Protein concentrations varied up to 2.62-fold (0.404 to 1.057 mg/mL), and Der f 1 contents varied up to 11.3-fold (3.597 to 40.688 μg/mL). Protein profiles of the extracts showed no major discrepancies, although there were some differences in SDS-PAGE band intensities, reflecting protein concentrations. Allergen potency ranged from 37038 to 60491 PAU/mL. The Prolagen product was highest in terms of protein concentration and allergen potency. The Lofarma product displayed Der f 1 content similar to that in Prolagen (19.4 μg/mg vs. 19.3 μg/mg). Endotoxin levels varied 8.9-fold (1020 to 8985 EU/mL). The newly developed house dust mite extract showed equal or better allergenic properties than available commercial extracts. This new product may be useful for better diagnostics and allergen-specific immunotherapeutics.

Key Words: Allergen, house dust mite, immunotherapeutics, standardization, potency

Allergy diagnosis and treatment depend on cross-reactivity between extracts from similar species, and allergenic properties may vary according to the environmental circumstances where an extract is produced. For the development of reliable allergy diagnostics and immunotherapeutics, standardization of allergen extract is a prerequisite.[1] However, in Korea, all commercial allergen extracts in clinical use are imported from the United States of America or European countries. In addi-

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tion, some commercial products are no longer available due to strengthened regulations.[2,3] Thus, in Korea, efforts have been made to standardize locally produced allergens since 2009 with support from the Korea Center for Disease Control and Prevention.[4] Previous studies have been performed for standardization of extracts for house dust mite (HDM),[5] cockroach,[6] pollens of regional importance,[7] insect venom component allergen (Pac c 3 from *Pachycondyla chinensis*),[8] and buckwheat.[9] The stabilities of extracts for HDM,[10] cockroach,[11] and pollens[12] have also been studied. Standardization of food allergens, including cereals and various regionally important fish species, is ongoing.

A company, 'Prolagen (Seoul, Korea)', established in 2017, obtained good manufacturing practice accreditation for the production of allergen extracts for allergy immunotherapeutics from the Ministry of Food and Drug Safety in 2019. Previously, eight skin prick test reagents produced from Prolagen were shown to be comparable to those of imported products.[13]

In this study, we compared the allergen extract for *D. farinae* produced by Prolagen with imported ones. Protein concentrations, major allergen contents, endotoxin levels, and potency among these commercially available products were compared by in vitro methods.

The Prolagen HDM allergen extract was prepared as described previously.[5] For comparison, HDM extracts were obtained from Jubilant HollisterStier Allergy (Spokane, WA, USA), Lofarma S.p.A. (Milano, Italy), and Stallergenes Greer (Boston, MA, USA). All extracts investigated in this study are manufactured as immunotherapeutic reagents, except the Lofarma product, which is for skin testing. Also, Stallergenes Greer's product is for veterinary use, not human use.

Protein concentrations were determined using a Bradford assay kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Concentrations of major allergens Der f 1 and Der f 2 were quantified using two-site ELISA kits from Indoor Biotechnologies Inc. (Charlottesville, VA, USA). Protein concentrations were determined to be 404–1057 μg/mL. Among the *D. farinae* products, the Prolagen product had the highest concentrations of Der f 1 and Der f 2, while the Stallergenes Greer product licensed for veterinary use had the lowest concentrations (Table 1). Der f 1 and Der f 2 content varied about 5.6- to 14.0-fold, respectively. The sum of these two major allergen contents differed by up to 6.9-fold, and the sum of these major allergen concentrations differed up to approximately 3.4-fold even after conversion into μg/mg protein concentrations. The ratio of Der f 1 to Der f 2 varied from 1:0.404 to 1:1.396 (3.5-fold) (Table 1). Der f 1/2 ratio may be affected by diet[14] and temperature.[15] Extraction method may also affect Der f 1/2 ratio.[5]

Protein profiles of the commercial extracts were compared by 15% SDS-PAGE (20 μL/well). Der f 1 and Der f 2 were detected with anti-recombinant Der f 1 monoclonal antibody (mAb) 3D7[16] and anti-recombinant Der f 2 mAb 2F38[17] as described previously.[5] IgE reactive components were probed with pooled serum samples (1:4 diluted). Serum samples were kindly provided by TRINA BIOREACTIVES (Naenikon, Switzerland). More intense bands of high molecular weight proteins were seen with the Hollister-Stier products, whereas additional strong bands were noted in the Prolagen product (Fig. 1A). The strongest IgE reactivity to a 14 kDa allergen, putative Der f 2, was shown by IgE immunoblot analysis in all examined extracts (Fig. 1B). Interestingly, relatively poor recognition of Der f 2 was observed for the Stallergenes Greer extract, even though similar recognition was shown with mAb 2F38. This is possibly due to polymorphism of Der f 2: mAb may recognize highly conserved regions, whereas human IgE antibodies recognize polymorphic residues. Weak IgE reaction to a 25 kDa component, putative Der f 1, was also displayed. MAbs strongly recognized Der f 1 (Fig. 1C) and Der f 2 (Fig. 1D) in the extracts. Degraded Der f 1 was detected in the Lofarma product (Fig. 1C).

A previous study used intradermal skin tests to determine the potency of HDM extracts[5] because this method is known to be more sensitive than skin prick tests.[18] However, we produced a new in-house reference preparation and carried out skin prick tests to meet the European standard.[19,20] A newly established potency was designated as Prolagen Allergy Unit (PAU). Competitive inhibition ELISA was performed, and allergenic potency was determined using 50% inhibitory concentrations of each allergen extract.[21] Allergen potencies differed up to about 1.6-fold, 37038–60491 PAU/mL (Fig. 2 and Table 2), which is similar to the sum of major allergen contents.

Endotoxin levels in extracts were measured using a kinetic *Limulus* amoebocyte lysate assay (Kinetic-QCL, Bio Whittaker, LONZA, Basel, Switzerland). Endotoxin levels in the extracts ranged from 1020–9110 EU/mL (up to 8.9-fold). Endo-

Table 1. Contents of *D. farinae* Allergen Extracts Commercially Available in Korea

Product			Protein concentration	Major allergen content					
				Der f 1		Der f 2		Sum (Der f 1 & 2)	
Manufacture	Lot No.	Licensed use	μg/mL	μg/mL	μg/mg	μg/mL	μg/mg	μg/mL	μg/mg
H, HollisterStier	E1900466	Human	523	3.6	6.9	3.5	6.8	7.1	13.6
L, Lofarma	190702	Human	569	11.0	19.4	6.7	11.8	17.7	31.2
G, Greer	363113	Veterinary	404	5.0	12.4	2.0	5.0	7.1	17.5
P, Prolagen	DF18001	Human	1057	20.3	19.3	28.4	26.9	48.8	46.1
	Average		638.3	10.0	14.5	10.2	12.6	20.2	27.1

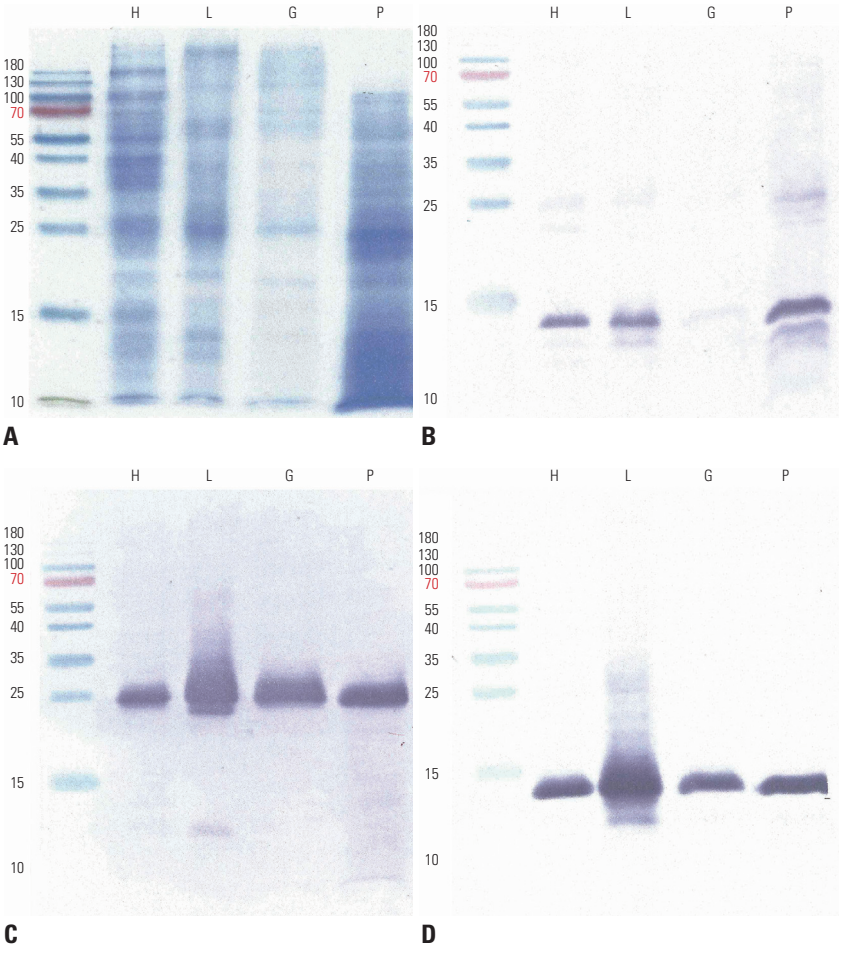

Fig. 1. Protein and allergen profiles of commercial *Dermatophagoides farinae* extracts. (A) Proteins (20 μL each) were separated onto 15% SDS-PAGE. (B) IgE reactive components were then probed with pooled patient sera. Der f 1 (C) and Der f 2 (D) allergens were detected with the use of monoclonal antibodies.

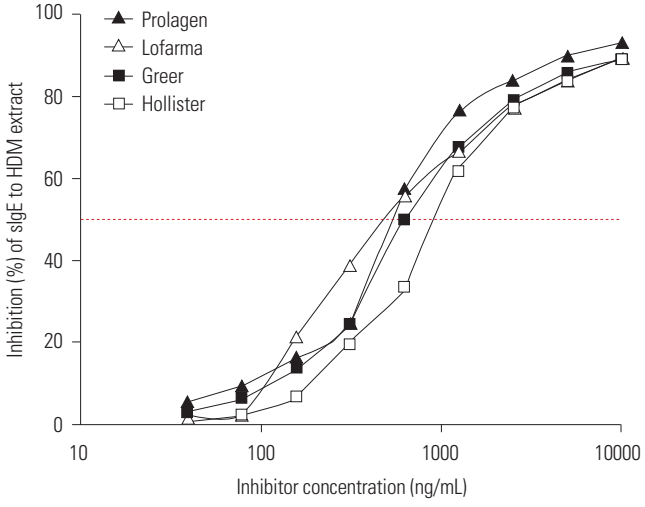

Fig. 2. Allergen potencies of commercial *Dermatophagoides farinae* extracts. Prolagen product with defined allergen units was coated, and IgE reactivity was inhibited with different commercial extracts to compare potencies.

Table 2. Comparison of Allergen Potencies of Commercial *D. farinae* Extracts

Product	Potency (Allergy unit)		
Manufacture	Provided	50% inhibitory concentration (ng/mL)	PAU/mL (PAU/mg)
H, HollisterStier	10000 AU/mL	916.8	37038 (46125)
L, Lofarma	100 DBU/mL	561.3	60491 (75332)
G, Greer	11000 PNU/mL	703.9	48244 (60081)
P, Prolagen*	150000 PAU/mL	580.9	58453 (72794)
Average		690.8	51057 (63583)

*Available as freeze-dried powder.

toxin levels per mg of protein differed by up to 6.9-fold (2524.8 to 17418.7) (Table 3). Endotoxin levels in commercial HDM extract vary up to 1000-fold (31 to 5200 EU).[22] The facultative intracellular bacteria *Bartonella* may be a mite symbiont and is thought to be the major source of endotoxin in HDM extracts.[23] Although difficult, endotoxin levels should be lowered to minimize possible side effects.

Varying composition and allergen content in commercial HDM allergen extracts commonly used for allergy diagnosis

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Table 3. Endotoxin Levels in *D. farinae* Allergen Extracts Commercially Available in Korea

Product	Endotoxin level	
Manufacture	EU/mL	EU/mg
H, HollisterStier	9110	17418.7
L, Lofarma	6110	10738.1
G, Greer	1020	2524.8
P, Prolagen	8985	8500.0
Average	6306	9795.4

and immunotherapy have been described.[24,25] Analysis of commercial allergen extracts of HDM may provide useful information for clinicians to select optimal products. Induction of blocking antibodies may vary according to the allergen products.[26] The IgE reactivity of allergens may be affected by not only species, but also by polymorphism of individual allergens, especially for Der f 2 and Der p 2.[27,28] Different sensitization patterns with different disease entities, respiratory or cutaneous allergies, also imply that careful product selection may influence the efficacy of immunotherapy.[29] For patients with respiratory allergy sensitized to HDM, better efficacy is expected with products containing extracts from raw materials with abundant mite feces, which contain more group 1 and 2 allergens. However, patients with cutaneous allergy are more likely to benefit from products with whole body extracts that may contain more minor allergens.

We analyzed the various commercial products licensed for immunotherapeutic, skin test, and veterinary use. In fact, these products were the only products we could obtain at the moment in Korea, although we hope to compare more products. It should be mentioned that the major allergen concentrations and potency of the reagents should differ in relation to the purpose of clinical application.

In conclusion, the *D. farinae* extract newly manufactured by Prolagen is comparable to imported products. This product may be useful for the development of better diagnostics and allergen-specific immunotherapeutics.

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AUTHOR CONTRIBUTIONS

Conceptualization: Ji Tae Kim, Kyoung Yong Jeong, and Jung-Won Park. Data curation: Ji Tae Kim, Kyoung Yong Jeong, and Jung-Won Park. Funding acquisition: Jung Dong Kim, Kyoung Yong Jeong, and Jung-Won Park. Investigation: Ji Tae Kim, Hyunho Kim, Sung Hyun Kim, Yeji Shin, Dong Jun Kim, Hangyeol Song, and Seok Woo Jang. Methodology: Kyoung Yong Jeong and Jung-Won Park. Project administration: Ji Tae Kim and Kyoung Yong Jeong. Resources: Jung Dong Kim, Kyung Hee Park, Jae-Hyun Lee, and Jung-Won Park. Supervision: Ji Tae Kim, Kyoung Yong Jeong, and Deug-chan Lee. Validation: Ji Tae Kim and Kyoung Yong Jeong. Writing—original draft: Ji Tae Kim, Kyoung Yong Jeong, and Jung-Won Park. Writing—review & editing: all authors. Approval of final manuscript: all authors.

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